J. New York Entomol. Soc. 93(3):1109–1112, 1985

ANTAGONISM OF ENTOMOGENOUS FUNGAL EXTRACTS TO THE DUTCH ELM DISEASE FUNGUS, *CERATOCYSTIS ULMI*

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Abstract.—Culture filtrates extracted from two species of entomogenous fungi, *Metarhizium anisopliae* and *Nomuraea rileyi,* and a commercial toxin, beauvericin, demonstrated antimycotic activity to two strains of the causal agent of the Dutch elm disease, *Ceratocystis ulmi.* Extracts of *Beauveria bassiana,* as tested, displayed no antagonism to *C. ulmi.* Beauvericin was antagonistic only to the aggressive strain of *C. ulmi,* while the entomogenous fungal species were antagonistic to both the aggressive and nonaggressive strains of *C. ulmi. M. anisopliae* produced the highest level of antagonism to *C. ulmi,* and the aggressive strain of *C. ulmi* showed greater sensitivity to antagonism than the nonaggressive strain. Extracts from both strains of *C. ulmi* produced no antagonism against the three species of entomogenous fungi.

The possible use of entomogenous fungi to control the insect vector of Dutch elm disease (DED) was supported by reports of fungal pathogenicity to elm bark beetles (Coleoptera: (Scolytidae) (Barson, 1976, 1977; Doane, 1959; Doberski, 1981). Agents used to control the insect vector may also interact secondarily with the fungal pathogen. Alternatives to chemical and classical biological control of *Scolytus multistriatus,* the insect vector of Dutch elm disease, must also take into account the possible antagonism between *Ceratocystis ulmi,* the causal agent of DED, and entomogenous fungi. Several species of nonentomogenous and entomogenous fungi have demonstrated antagonism to both the aggressive and nonaggressive strains of *Ceratocystis ulmi* (Gemma et al., 1984; Gibbs and Smith, 1978; Holmes, 1954; Webber, 1981). To determine the basis for this activity, culture filtrates of three species, *Beauveria bassiana, Metarhizium anisopliae,* and *Nomuraea rileyi* were prepared and their antimycotic activity tested against *C. ulmi* along with beauvericin, the commercially prepared toxin from *B. bassiana.*

METHODS

Culture filtrates from *Beauveria bassiana* (Bals.) Vuill. ATCC18514, *Metarhizium anisopliae* (Metsch.) Sorokin ATCC22099, and *Nomuraea rileyi* (Farlow) Samson (obtained from C. Ignoffo, USDA, Columbia, Missouri) were evaluated for their capacity to inhibit the growth of two strains of the DED fungus, *Ceratocystis ulmi* (Schreiber and Addison original strain) aggressive (A) and *Ceratocystis ulmi* nonaggressive strain (NA). The cultures were incubated in 250 ml Erlenmeyer flasks containing 30 ml potato dextrose broth (PDB) (with the exception of *N. rileyi* which was grown on Sabouraud's maltose broth plus 1% yeast extract) at ambient temperature in complete darkness. For each species, 30 flasks were inoculated with an agar

Table 1. The effect of time and concentration on the inhibition of two strains of *Ceratocystis ulmi* by filtrates of cultures of entomogenous fungi.

Age of culture when filtrate was extracted	2 weeks		3 weeks		4 weeks		4 weeks	
Filtrate concentration (W/V)	3.2%		3.2%		3.2%		6.4%	
Strain of *C. ulmi*	A	NA	A	NA	A	NA	A	NA
Culture filtrate								
B. bassiana (ATCC18514)	+[a]	+	+	+	+	+	−	−
M. anisopliae (ATCC22099)	106[b]	89	110	88	106	89	181	121
N. rileyi (Missouri strain)	+	89	109	+	99	124	148	116

[a] + = slight inhibition; − = no inhibition.
[b] = zone of inhibition (mm^2).

plug (7 mm diam) cut with a flamed cork borer from the surface of a 48 hr. mycelium on water agar. Culture filtrates were then collected at intervals of two, three, and four weeks after inoculation.

Culture filtrates were prepared by filtering the contents of 10 flasks for each fungal species through a Nalgene® Sterilization Filter Unit (pore size 0.45 μm), and dehydrated by freeze-drying. The residue was dissolved in sterile 0.01% Triton-X solution to produce a concentration of 3.2% (W/V). Uninoculated aliquots of culture broths were treated similarly and used as controls. The residue from the filtrate collected at four weeks was reconstituted to produce two final filtrate concentrations of 3.2% and 6.4% (W/V).

For the evaluation of filtrate activity, a mycelial mat of *C. ulmi* (strain A or NA) was homogenized in 30 ml of sterile 0.01% Tween 80 solution in a sterile Waring blender. One ml aliquots of the slurry were then spread on the surface of appropriate agar medium in a 100 × 15 mm Petri dish. Four wells (1 × 8 mm) were cut in the agar surface of each plate with a sterile cork borer and 0.1 ml sample of a given filtrate was transferred to each of three wells; an appropriate control was placed in well 4. Five replicates were made for each filtrate tested. Zones of inhibition, if produced, were measured at the end of four days. The antimycotic activity of culture filtrates of *C. ulmi* (A and NA) was determined similarly. Beauvericin (Sigma Chemical Co. B7510) dissolved in methanol at a concentration of 0.5 mg/ml (W/V) was tested in the same manner against *C. ulmi* (A and NA).

RESULTS

Antimycotic activity against both strains of *C. ulmi* was demonstrated by culture filtrates produced from *M. anisopliae* and *N. rileyi,* respectively (Table 1) (Fig. 1). Filtrates from older cultures did not produce greater zones of inhibition when compared with those of filtrates from younger cultures, but doubling the concentration of the four week old filtrates from *M. anisopliae* and *N. rileyi* did significantly increase the zones of inhibition (Table 1). Extracts from *M. anisopliae* cultures produced the highest level of inhibition against both strains of *C. ulmi* with the A strain more susceptible. *C. ulmi* extracts (A and NA) produced no antimycotic activity against

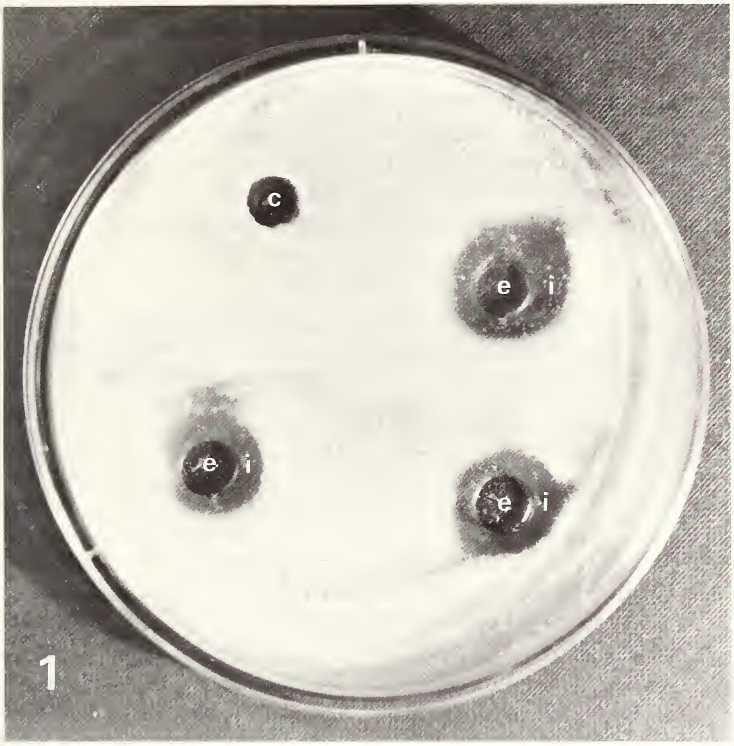

Fig. 1. The aggressive strain of *Ceratocystis ulmi* growing on the surface of potato dextrose agar in a Petri plate. Three of the wells (e) contain a filtrate (concentration 6.4%) of a four week culture of *Metarhizium anisopliae* and are surrounded by a zone of inhibition (i). The control well (c) contains a filtrate prepared from sterile liquid medium.

B. bassiana, M. anisopliae, and *N. rileyi.* Beauvericin produced a slight inhibition zone against *C. ulmi*-NA, but an inhibition zone of 110 mm^2 resulted in tests against *C. ulmi*-A.

DISCUSSION

Fungal extracts of two entomogenous culture filtrates showed clear antimycotic activity against *C. ulmi*; furthermore, the activity increased with the concentration. Failure of the filtrate from four week old mycelia to produce a larger zone of inhibition than the filtrate from two week old mycelia seems to indicate that antimycotic substances are not continuously produced during mycelial growth in culture. Although several studies have reported the isolation of several different metabolites from *C. ulmi,* including phenolics, phytotoxic glycopeptides, and a wilt-inducing toxin, ceratoulmin (Claydon et al., 1974; Strobel and Lanier, 1981; Takai, 1974), culture extracts of *C. ulmi* did not demonstrate any antimycotic activity to those species of entomogenous fungi tested. These results support the potential of these entomogenous

fungi as biological control agents of the pathogen of DED as well as for control of its vector, the elm bark beetle.

Differences between the aggressive and nonaggressive strains of *C. ulmi* in response to fungal extracts and beauvericin may result from each strain's genetic differences which alter their response to external factors (Pusey and Wilson, 1979). Additionally, the effect of such factors as strain variation, temperature, degree of aeration, nature of the medium, and moisture content of the medium (Ciegler, 1977) on the production of antimycotic substances by entomogenous fungi needs to be examined carefully.

ACKNOWLEDGMENTS

This research was supported in part by a Cooperative Agreement between Rhode Island College and the USDA Forest Service, Northeast Forest Experiment Station, Broomall, Pennsylvania. A grant from the Faculty Research Fund, Rhode Island College, is also gratefully acknowledged.

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Received March 14, 1984; accepted July 24, 1984.